Animal Model of Mild Traumatic Brain Injury: An Assessment of Impact Location

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Abstract

With a high prevalence of mild traumatic brain injuries that occur in sports today, more research is needed in order to reveal the biomechanics behind these devastating and often life-changing injuries. At the professional level, the high paced nature of football places athletes at an increased risk of brain injury as concussion rates have increased over the years. New safety protocols have put in place in an effort to curb the rate of head injuries, but more research is required in order to enhance these safety protocols. In this study, a rodent concussion model was created in order to study the effects of concussions in different locations of the head. Using a controlled cortical impactor device, identical concussive impacts were applied to 2 different locations (top and back) on the head of Sprague Dawley rats (n = 24). Balance and memory were assessed before and after the impact using angle board and novel object recognition tests respectively in order to determine the severity of the concussive impacts. Unpaired t-tests for balance testing revealed a p-value of 0.00029414 for impacts to the top of the head, revealing a significant decline in balance post-injury for this group of animals while the unpaired t-tests for impacts to the back of the head revealed a p-value of 0.1852, indicating no significant change in balance. Memory tests were unable to find significant changes in memory post-injury. Statistically, a head injury was successfully produced at the top of the head, but not at the back. This may indicate that injuries to the top of the head are more susceptible to concussion than the back, but further studies involving more sensitive testing methods of balance and memory are required in order to statistically prove a significant difference between the two locations.

Introduction

Concussions, or mild traumatic brain injuries (mTBI), are head trauma-induced injuries defined by altered levels of brain function with or without loss of consciousness (LOC) (21).

Approximately 1.54 million of these head injuries occur each year. About 20% of these cases occur during sports with 9% of these sports-related injuries being diagnosed as concussions (14). The high paced nature of football, especially at the National Football League (NFL) level places players at a high risk of injury. Approximately 5.4 concussions were reported by ESPN's *Outside The Lines* per week in the 2009 season. These numbers jumped up to 7.6 per week in 2010 and 8.4 in 2011 (18). Much research has been done in the field of concussions in an attempt to better understand its ambiguity and complexity. As more research is conducted, awareness of the future implications of concussions has risen to higher levels (19). New research has led to the mandating of concussion protocols by major sports leagues in an attempt to help reduce the long term effects of this relatively unknown condition (22). One of these devastating long term effects is gradual degeneration of the brain as a result of repeated head trauma; a condition known as Chronic Traumatic Encephalopathy (CTE). CTE has been linked to high impact sports such as football and its effects on the quality of life are severe. Individuals posthumously diagnosed with the disease showed signs of dementia, memory loss, and depression in latter stages of life (20).

Background/Literature Review

Though concussions can sometimes be identified through the presence of different symptoms, the heterogeneity of the human brain and inconsistency of brain response to trauma can make it difficult to assess the level of damage and neuropsychological outcomes for different individuals. The current goals of mTBI research include improving methods of concussion detection and diagnosis. For sports such as football where the magnitude of impacts can now be measured through helmet based sensors, these methods could potentially include identifying a concussion "threshold of injury," which would determine a minimum amount of force or a range of forces and resultant head acceleration caused by an impact to the head that is likely to result in a concussion through a series of related head measurements such as velocity and acceleration (1).

Concussions are a type of closed head injury. Closed head injuries involve a blunt force applied directly to the head without fracture of the skull or the dura mater. The force applied to the head creates linear and rotational accelerations within the brain depending on the position of the impact relative to the head center of mass, and this resultant acceleration causes further injury to the brain in addition to the impact itself (15). This is why brain injuries can occur without direct impact, but by an indirect impact that creates acceleration forces within the brain (e.g. a car crash jerking the head without direct contact of the head to any object). Which type of acceleration (linear or rotational) is more harmful is under debate. Studies have provided arguments for both cases. (14) (15) (23) (24).

In 1994, the NFL created a committee to study mTBI sustained by professional football players between the years 1996-2001 (8). Through analysis of game video and reconstruction of concussive NFL impacts using Hybrid III test dummies, the committee was able to label several observed parameters based on the video: impact velocity, severity index (SI), head injury criterion (HIC), head translational acceleration, head change in velocity, head rotational acceleration, head rotational velocity, and impact force (11). Using this information, they were able to identify the average measurable biomechanical responses present in these impacts that were shown by collected video data to produce concussions (the impact velocity, etc.).

In 2004, Simbex developed an impact sensor technology known as the Head Impact Telemetry System (HITS) that was implemented in Riddell brand football helmets (12). The sensors, imbedded within the helmet, would pick up head impact information and transmit this data wirelessly to a sideline laptop which could be monitored by the athletic trainers/medical staff. The sensors detect parameters similar to those obtained from the NFL's video analysis such as impact force, duration of impact, and resultant accelerations. By making connections between the recorded impact parameters and the concussion diagnoses, the likelihood of concussion can possibly be determined (11). Although this technique is not yet considered a diagnostic tool due to the differences in brain response between different individuals, studies with this technology have been conducted on human subjects in order to better understand the mechanics of concussion, and how and what differences between people affect brain response to concussive impacts (2). In a study of high school football players using the newly developed HITS software, Broglio et al. identified several factors that can affect the acceleration of the head following an impact. The results showed differences between various body and head masses. Location of the impact was also a major factor. Frequency of hits to each head location is dependent upon the position being played (2). The top, front, back, left, and right side are common azimuth locational descriptions of the head. Hits to the top of the head produced the greatest linear accelerations, followed by hits to the front, back and then sides (1). Though this was the case, hits to the top of the head also received a greater average impact force than the other locations, so it is unknown whether or not the resultant accelerations and resultant brain response would have been the same had the forces been equal for each location.

Animal models of brain injury have been used to analyze the results of various types of head trauma. Many different techniques and devices have been implemented such as the weight-drop, cortical impact, and fluid percussion models, with the goal of creating a reproducible model of head injury that can eventually be applied to more upscale beings (e.g. larger animals, humans). For simulating concussions in animals, research has determined that the closed head injury model is a good model for the application of head trauma (16). After scaling the forces encountered by humans down to animals such as laboratory rats, analyses of head impacts have provided insights into the biomechanics, pathology, and behavioral effects of brain injury. Scaling has involved comparisons between dimensions of the human and rodent brain such as the longitudinal radius, and application of equations that relate these two dimensions. For example, Guttierez et al. used the following equations to scale head acceleration from a human to an animal (30):

1. $$\lambda = \frac{longitudinal\ radius\ of\ human\ brain}{longitudinal\ radius\ of\ animal\ brain}$$

2. $$a_{animal} = a_{human}(\lambda)$$

λ = scaling factor
a = acceleration

These equations are based on the principle that a smaller brain requires more acceleration to produce similar injury seen in animals with larger brains (30).

In 1994, Anthony Marmarou designed an animal model of lethal closed head injury using a device called the drop-weight impactor. This is one of the most widely regarded models of diffuse brain injury in the rat (4). It evaluated brain response to trauma through application of force to the top of the rat head at midline, but with fracture to the skull. Though the method was later corrected to remove skull fracture by providing a protective disk that essentially acted as a helmet, evidence of skull deformation was still present, which raises suspicion that parts of the injury were caused by direct contact between the impactor and the brain through the skull (6). Due to these limitations and the fact that the impacts were not of the same caliber as those generally experienced in football (the forces were much more severe with a significant mortality rate as a direct result of the impact), these studies do not present animal models of brain injury that are accurately representative of head trauma in the sport of football.

A study done by Kilbourne et al., known as the Maryland model, assessed severe impacts to the frontal region of the rat brain. This study claims that impact results are dependent upon the anatomical site that the force is applied as well as the axis of application (4). It utilized a different method of frontal impacts by applying a force horizontally through two beams attached to the front of the rat head around the nose, which produced anterior-posterior linear acceleration. The length of one of the two beams was 3mm longer than the other, which induced rotational acceleration in the sagittal plane. The Marmarou model applied a force to the top of the head, which produced dorsal-ventral linear acceleration. The results of the Maryland model study show significant differences in comparison to the Marmarou model in terms of pathology since the areas affected by each hit were different. There was evidence of subarachnoid hemorrhage behind the cerebellum in 10/25 rats tested. Petechial hemorrhages were also common in white matter in the frontal and parietal lobes, in the rostral corpus callosum, in the deep nuclei, and in the brainstem. Some blood clots formed in veins as well (4). The rats in the Marmarou study had signs of edema in the thalamus and brainstem. There were severed axons in the brainstem, and axonal abnormalities in the optic tracts, rubrospinal tracts, and corticospinal tracts in the pons and medulla oblongata. The rats also had significant damage immediately beneath the site of impact which may have been attributed to the slight skull deformations caused by the impactor. (6) Though the magnitude of the impacts were much larger in the Marmarou model, the fact that different brain structures were affected in each model supports the idea that brain response from an impact is dependent upon the anatomical site of impact and the axes that the resultant impulse travels along.

A study done by Viano attempted to create a true animal model of injury in the NFL by applying forces through a type of ballistic impact device that was specially designed by the investigators. It is much easier to test head impacts on animals and by creating an animal model that can be translated to humans, the model can be used in further studies to answer a variety of different questions that couldn't possibly be answered through human testing (e.g. the effect of multiple concussive impacts to the head within a certain time frame). The Viano model derives its concussion parameters from video analysis and impact re-creation (8). The study includes the use of a protective helmet in order to help produce the proper accelerations and impact durations without skull fractures. It claims to apply impact conditions as seen in the NFL, but the derived data is obtained from their own studies, which has been speculated of being biased towards more injurious impacts (3). Also, there is speculation that the dummies used to recreate the impacts from game-play video were not accurate with respect to properly scaled head mass, body mass, and neck stiffness which may have led to errors in the mechanical data (3). The study is self-fulfilling in that the investigators readjusted the thickness and stiffness of the helmet in order to produce the "correct" biomechanical response, which renders the point of applying a high velocity impact obsolete. The impact is applied to the left side of the head in a "standard position." If the impacts were to be applied to a different location on the head, it is speculated that the biomechanical response would be different and the helmet would have to be further readjusted in order to produce the same data. Nevertheless, the parameters it uses are at least somewhat relevant in terms of representing concussive impacts in football, and the animal model was able to closely reproduce this biomechanical data with pathological results similar to those seen in humans following concussion (8).

Creed et al. created a model of concussion in mice through the use of a controlled cortical impactor (CCI) device (16). Closed head impacts were performed on mice at the midline between lambda and bregma (top impact) which caused minor skull fracture. Post-injury testing revealed deficits in spatial learning and working memory within the first 3 days of injury. These deficits were all resolved within 4 days of the impact. Swelling and degeneration of axons were also noted along with neuronal dysfunction which most likely contributed to the observed behavioral deficits.

Previous models have limited impacts to a specific area since these studies attempt to recreate a specific type of injury where impacts only occur to one region of the head (example: car crashes usually result in frontal impacts), creating head acceleration in one specific plane, but impacts in football can occur from many different angles to different sides of the head. It is known that damage done to the brain is not only dependent upon the force of impact, but on the anatomical location, axis of application, and angle of application (4) (15). Forces applied to different parts of the head will produce different types of resultant accelerations along different planes (e.g. impacts applied directly to the front of the head will produce anterior-posterior linear acceleration whereas impacts applied directly at the top of the head at midline will produce dorsal ventral linear accelerations) and will have varying effects on brain structures (4). In 1779, a surgeon named Percivall Pott made a simple connection between the location of an impact and the severity of the brain injury. He noted that patients with frontal impact brain injuries recovered quicker than those who received injuries to other parts of the head (15). Though the impact forces on those patient's heads and the other impact locations are unknown, this implies notable differences in brain response depending on the location of impact. Hicks et al. identified a significant correlation between memory following traumatic brain injury and the amount of neuronal loss in the dentate gyrus of the hippocampus (17). The location of an impact will theoretically have various effects on structures such as the hippocampus and how much neuronal loss occurs, therefore resulting in score variations on assessments such as those that test memory as well as those that examine other behavioral aspects that are linked to other brain structures affected by the impacts.

Model	Marmarou	Maryland	Viano	Creed et al.

Device	Drop-Weight Impactor	Rolling Ball/Dual Beam Impact Device	Ballistic Impact Device	CCI
Impact Severity	Severe	Severe	Mild	Mild
Impact Location	Top (Midline between Lambda and Bregma)	Front	Left Side	Top (Midline between Lambda and Bregma)
Fracture? (Y/N)	Y	N	N	Y
Observed Deficits	Hemorrhage, contusions, axonal damage.	Cerebellum hemorrhage, blood clots, severed brainstem axons, axonal abnormalities	Petechial hemorrhage, focal contusions	Spatial learning and working memory deficits. Axonal swelling and degeneration.

Table comparing the various models of Traumatic Brain Injury

Proposed Study/Motivation

The current study attempted to create an animal (rat) model of closed head injury with the overall goal of identifying the differences in brain response based on location through application of identical impacts to different locations on the head. It simulated impacts in football by applying a concussive impact to two different anatomical locations (top and back) on the animal head without causing skull fracture (closed head injury). The locations for injury on the rat head were identified by anatomical comparisons between the human and rat. A CCI was used to induce a closed head injury in the rat at the pre-determined locations. CCI devices are able to accurately reproduce head injuries in small animals as noted in previously reviewed literature. The impact was applied through adjustment of the CCI's velocity and depth settings. The study consisted of two parts. The first part attempted to inflict a concussion to the animal without fracturing the skull (identification of speed and depth of impact for the cortical impact device). The second part of the study consisted of applying concussive injury to a different location on the head using the parameters for concussion found in the first study, and then assessing the differences in brain response based on location of injury. Current animal models of concussion do not stress the importance of location on the resultant effects of mTBI on the brain. Though it is impossible to test an impact to every part of the head and every angle of impact, by applying a force to a few locations of the head and making assessments of cognition, pathology, and/or behavior, the relative vulnerabilities of these generic locations to an impact can be determined (Ex: a force to the top of the head may produce a more severe level of concussion than that same force applied to the back of the head). For the sake of time, this study examined two locations of the head. Future studies may analyze other locations. With the number of concussions in high impact sports such as the NFL increasing with each year, it is imperative that more is revealed about this relatively unknown condition (18). This study could possibly help lead to improvements in safety equipment, and may facilitate the credibility of impact software such as HITS as sideline assessment tools since these devices detect the locations of impacts. At the very least, it should improve the current understanding of the biomechanics of concussion.

Hypothesis

It is hypothesized that impacts to the back of the rat head will produce more sensorimotor deficits in comparison to impacts to the top of the head. This is due to the locations of the cerebellum and pons being closer to the back of the head and their involvement in regulation of balance and memory.

Materials and Methods

Impact Force and Injury Device

Note: All animal procedures will be approved by the Georgia Tech Institutional Animal Care and Use Committee (IACUC) (29)

The proper impact parameters required to induce concussion in the animal were determined through analysis of concussive impacts in football studies and the results of previous rodent models of injury. Based on previous models, an impact speed of 5 m/s at a depth of 1.5mm (using the cortical impact device) was deemed as the starting parameter for inducing concussion in the rat (16). For this study, adult male or female Sprague Dawley rats weighing between 225g and 350g were used. 24 rats were used in the study (7 per location and 5 shams per location). 3 more rats were used in practice and training procedures.

Cortical Impact Device: The CCI was used to produce a closed head injury on the rat. The device consists of a metal tipped impactor that moves and strikes the animal at the desired location using an adjustable velocity and depth.

Impact Force: The following is a rudimentary force analysis given the initial impact parameters of 5 m/s impact speed and 1.5 mm impact depth.

$$F = ma = \mathrm{m}\frac{\mathrm{V_f}^2 - \mathrm{V_i}^2}{2\Delta d}$$

a = acceleration of object
V_i = initial velocity of object
V_f = final velocity of object
d = distance (depth of impact)

$$F = .002kg\frac{[(0)^2 - (5m/s)^2]}{2(.0015m)} = 16.67\ N$$

These impact parameters and corresponding force were used initially, but were adjusted later in an effort to avoid skull fracture. Once the impact parameters (speed and depth) necessary to produce a concussion without skull fracture were identified at one site of the head, the same parameters were used at the other location since the impact force is identical if the speed and depth are kept constant.

Determining the sites of impact

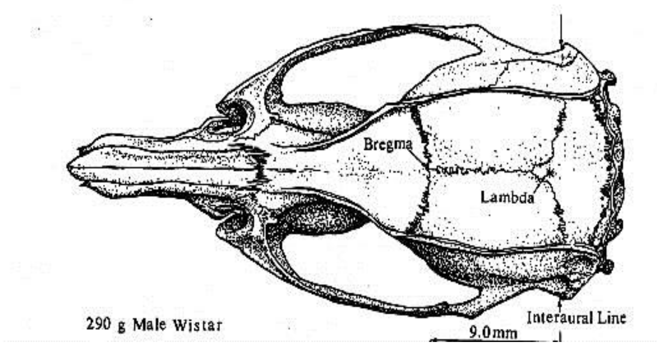

Figure 1: The major landmark positions of bregma and lambda on the rat skull are shown in the picture above

The impact locations for this study consisted of the front and back side of the head. The locations were scaled from the human head to similar anatomical regions on the rat head. The impacts are as follows:

Top - For impacts to the top of the head, the impact was placed around the middle of the rat skull approximately 2 mm posterior to bregma. (-4.5 mm Bregma)

Back - For impacts to the back of the head, the impact struck the occipital bone of the rat skull directly vertical at lambda. (-7.5 mm Bregma)

Preventing skull fracture – A protective object placed on the rat skull that would act as a "helmet" in order to prevent skull fracture was prepared in case the impacts did produce fracture. Skull fracture would induce further injury to the brain that is not present in concussion. Ultimately, the helmet was not used for the study.

Alternative Method: If skull fracture is initially found to not be present in helmetless impacts, then the helmet will be removed from the study.

Impact Procedure

Anesthesia: For all surgical procedures, the animals were anesthetized with isoflurane. Animals were placed in a plastic box that was fed with isoflurane to initiate anesthesia. During the surgical procedure, a mask that feeds isoflurane was placed over the nose/mouth.

Surgery Preparation: Following anesthesia, the animals were shaved over the head and body temperature was monitored and kept warm with heating pads throughout surgery. Preparation also included povidone-iodine, eye ointment, and lidocaine or marcaine at the incision site.

Brain Injury: The shaved scalp was swabbed with povidone-iodine and a subcutaneous injection of lidocaine was administered at the incision site. The animal was placed underneath the cortical impact device powered by compressed air and electronically controlled. The animal was strapped snug around the shoulder to minimize unnecessary movement of the body while the head was supported by a soft foam pad as it leveled with the body. The impactor tip (diameter of 3mm) was repositioned depending on the type of hit for that particular animal (front, top, back). The skull was expected to deflect and not fracture; any animal with skull fracture was removed from the study. The animal then received injury in one of the three locations. Following injury, the animal was placed on a heating pad while recovering from the anesthesia.

Analysis

In order to assess concussion in the rat, the following behavioral tests were performed.

Balance: The behavior and memory of the rats was tested for detection of sensorimotor deficits. An angle board behavior test was used to assess balance (25) (26). Balance is an acute measure of mTBI and concussion in humans. A rectangular plane (approximately 60 cm x 120 cm with an analog protractor and hinged base) was set at a starting angle (determined based on an initial assessment of rat balance ability) and each rat was placed on the board facing the upper edge at a distance approximately 10 cm from the top (in order to make sure that the rat does not use its tail to balance itself). They were held in place for approximately 5 seconds to allow them to establish stable footing and then released. If the rat did not slide backward down the inclined plane within 5 seconds, the trial was scored a success. The board incline angle was then increased (by 1° for maximum sensitivity). 4 trials per angle were given to each animal, with a 5 minute rest period between angle changes. Testing was discontinued if the rat failed to maintain stability on two consecutive angles. After another 5 minute resting period, the angle of failure was tested another 4 times to ensure that the failure was not by chance (i.e. 8 consecutive falls for increased sensitivity). The angle of the first fall, the total number of falls, and the threshold angle (defined as the last angle at which the rat succeeded by maintaining balance for 5 seconds in at least 2 out of 4 trials) were recorded.

Memory: Novel Object Recognition testing was also performed where, before injury, the rat was placed in an area containing two objects and explored and familiarized with those objects (27). After injury, one of the objects was replaced with a new one. If the rat spent significantly more time with the new object than the old one, cognitive function was considered intact. If it spent equal amounts of time exploring each of the two objects, then cognitive function was considered declined. This is based on the premise that rodents will spend more time exploring unfamiliar rather than familiar objects.

Results

Study 1: Inducing Concussion in the Animal Model

The purpose of the first study was to find the necessary cortical impactor device parameters that induce measurable concussive injury in the rat. 12 rats were used in this preliminary study. The initial parameters were set at a speed of 5 m/s and a depth of 1.5mm with dwell time of 0.5 seconds. The parameters were later adjusted to 5 m/s at a depth of 1mm following a trial run of the device and assessment of injury mechanics. The location of the impact was -4.5 mm Bregma (Figure 2) and was labeled as an injury to the top of the head. [Areas affected: Retrosplenial granular cortex, Retrosplenial dysgranular cortex (episodic memory)]

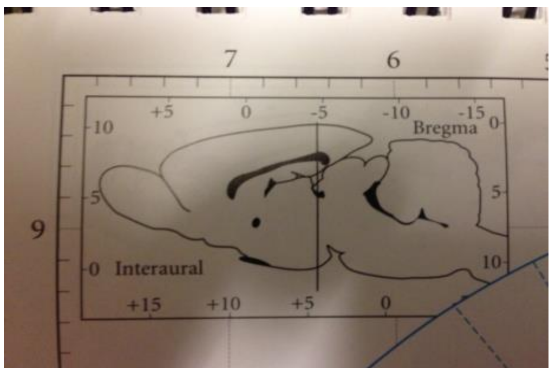

Figure 2 – Top impact at -4.5 mm in reference to Bregma. Solid vertical line indicates location.

Prior to injury, angle board measurements and Novel Object Recognition tests were run. The angle board test was performed twice in order to reduce learning effects. The test was performed a few days before the surgery/impacts and then performed right before the actual surgery and impacts took place. These tests were performed at consistent times of the day. After all 12 surgeries and impacts were performed, the angle board test was used again 4 hours post-operation to look for any signs of decline in balance.

Rat #	Pre-injury angle of failure	Post-injury angle of failure	Sham?
1	134 (46°)	137 (43°)	NO
2	134 (46°)	136 (44°)	YES
3	132 (48°)	132 (48°)	YES

4	134 (46°)	138 (42°)	NO
5	133 (47°)	134 (46°)	YES
6	133 (47°)	135 (45°)	NO
7	133 (47°)	132(48°)	YES
8	135 (45°)	140 (40°)	NO
9	134 (46°)	136 (44°)	YES
10	134 (46°)	137 (43°)	NO
11	135 (45°)	140 (40°)	NO
12	132 (48°)	138 (42°)	NO

Injury Group	Sham Group
Matched Pairs t-test	Matched Pairs t-test
p-value = 0.00029414	p-value = 0.242
$p < \alpha$ ($\alpha = 0.05$)	$p > \alpha$ ($\alpha = 0.05$)
Standard Deviation: 1.4142	Standard Deviation: 1.3038
Significant difference between pre-injury and post-injury groups.	NO significant difference between pre and post injury sham groups.

Injury vs. Sham	
Unpaired t-test (student's t-test)	Unpaired t-test (student's t-test)
Pre-injury	Post-injury
p-value = 0.280	p-value = 0.0055
Injured and Sham animals have relatively consistent failure angles pre-injury.	Injured group had significantly lower angle of failure than sham group for post-injured group.

The results of the angle board test for impacts to the top of the head are displayed in the table above. A matched pairs t-test was used to compare the differences in average angle measurements between pre and post injury groups. Statistical analysis was performed using MATLAB version 7.10.0.499 (R2010a). A Kolmogrov-Smirnov goodness of fit hypothesis test confirmed that data follows a normal distribution. All subjects survived the surgical procedure and corresponding data were included in the results. Matched pairs t-test of the injury group revealed a significant decline in balance ability for the rats. Analysis of sham groups revealed no significant change in balance ability. Unpaired t-tests were also used to measure consistency between animals used in sham and injured groups. Analysis revealed consistent balance ability between both groups of animals. These results indicate successful application of injury to the animal based on balance scores.

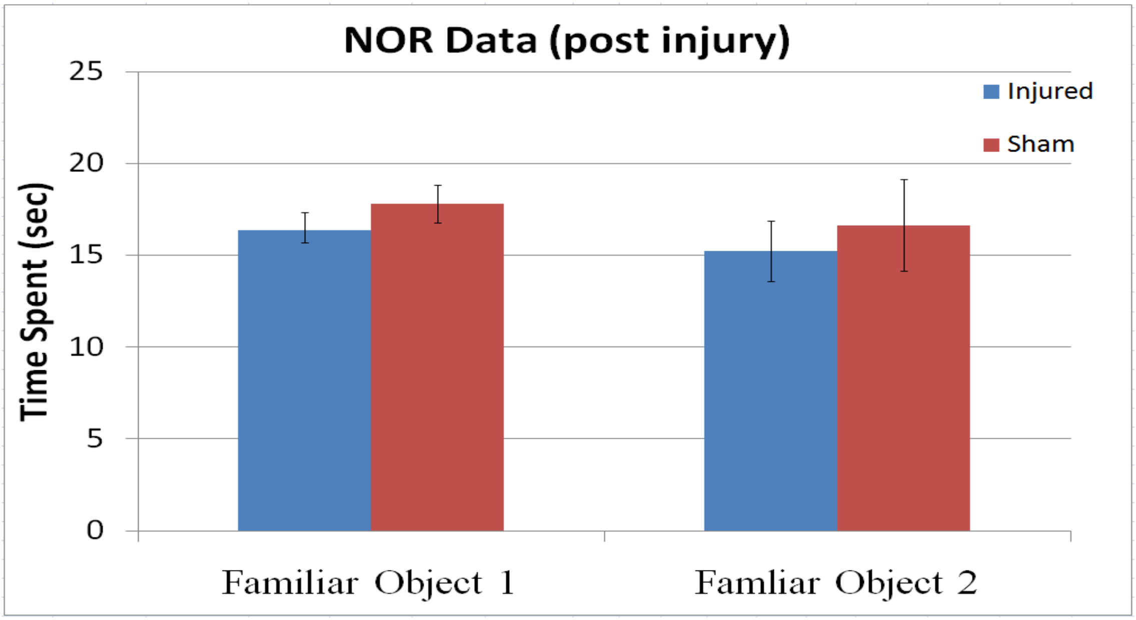

Figure 3: Time Spent Observing Two Familiar Objects

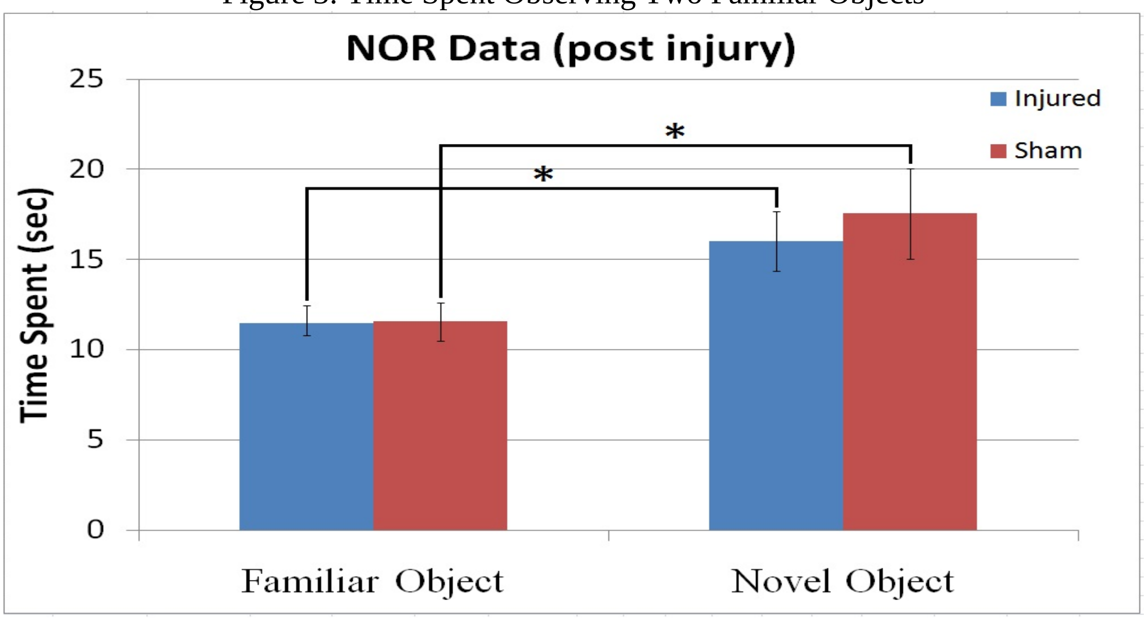

Figure 4: Time Spent Observing Familiar Object and Novel Object

The above graphs show the results of the Novel Object Recognition Memory test. The data did not show any significant deficits in memory after injury. According to Figure 3, there was no significant difference in the amount of time spent exploring two identical objects. According to figure 4, there was significantly more time spent exploring a novel object for both the sham and the injured groups (if deficit is present, injured group should spend an equal amount of time exploring both familiar and novel objects).

Based on the assessment of rat balance, it was determined that a deficit in brain function was achieved using the parameters listed above. Identical parameters will now be used to apply impacts to the back of the head.

Study 2: Effects of Location on Brain Response to Concussion

Following the impacts to the top of the head, the impact coordinates were changed to simulate impacts sustained to the back of the head at -7.5 mm bregma (Figure 5). [Areas affected: retrosplenial dysgranular cortex (episodic memory), retrosplenial granular cortex, pineal gland, secondary visual cortex mediolat and mediomed, intermediate gray layer SC (superior colliculus) (deals with vision)]

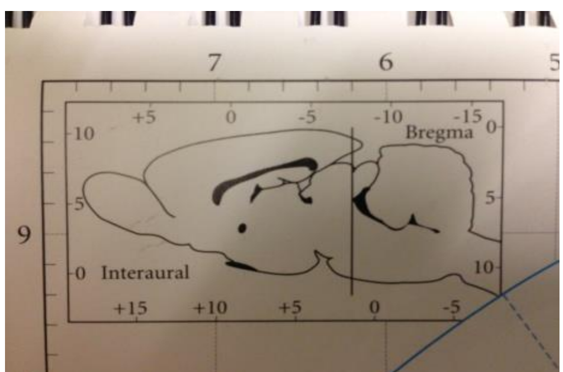

Figure 5 – Back impact at -7.5 mm in reference to Bregma. Solid vertical line indicates location.

The same procedures were used from Study 1.

Rat #	Pre-injury angle of failure	Post-injury angle of failure	Sham?
1	135 (45°)	DIED	NO
2	132 (48°)	132 (48°)	YES
3	132 (48°)	133 (47°)	YES
4	132 (48°)	135 (45°)	NO
5	133 (47°)	134 (47°)	YES
6	133 (47°)	135 (47°)	NO
7	134 (46°)	134 (46°)	YES
8	135 (45°)	134 (46°)	NO
9	134 (46°)	134 (46°)	YES
10	130 (50°)	131 (49°)	NO
11	132 (48°)	133 (47°)	NO
12	131 (49°)	132 (48°)	NO

Injury Group	Sham Group
Matched Pairs t-test	Matched Pairs t-test
p-value = 0.1852	p-value = 0.3739
$p > \alpha$ ($\alpha = 0.05$)	$p > \alpha$ ($\alpha = 0.05$)
Standard Deviation: 1.3292	Standard Deviation: 0.4472
NO Significant difference between pre- treatment and post- treatment experimental groups	NO significant difference between pre-treatment and post-treatment sham groups

Injury vs. Sham	
Unpaired t-test (student's t-test)	Unpaired t-test (student's t-test)
Pre-injury	Post-injury
p-value = 0.3663	p-value = 0.7881
Injured and Sham animals have relatively consistent failure angles pre-injury.	Injured and Sham groups had no significant difference in failure angles post-injury. Neither group of animals (injured or sham, had significant declines in failure angle)

The results of the angle board test for impacts to the back of the head are displayed in the table above. A matched pairs t-test was used to compare the differences in average angle measurements between pre and post injury groups. Statistical analysis was performed via MATLAB version 7.10.0.499 (R2010a). A Kolmogrov-Smirnov goodness of fit hypothesis test confirmed that the data follows a normal distribution. Subject number 1 of the injury group did not survive the surgical procedure; therefore the corresponding data was not recorded leaving 6 experimental test subjects and 5 shams. Matched pairs t-test revealed no significant changes in balance ability of the injury group before and after injury. Matched pairs t-test of the sham group revealed no significant change in balance ability. An unpaired t-test of sham and injured animals was conducted to ensure equal balance ability between animals before injury. Analysis revealed equal balance ability between the two testing groups. Unexpectedly, the same impact to the back of the head was unable to produce changes in balance ability of the rat. A high standard deviation value of 1.3292 indicates high variability between data and may be due to outliers in the study.

Discussion

Statistical analyses indicate that creating mTBI in the rat with an impact to the top of the head was successful in terms of inducing concussion symptoms, but was unsuccessful for the back of the head although identical impact parameters were used for both locations. It was hypothesized that different locations of impact would affect different anatomical structures of the brain. If a structure of the brain was involved in concussion metrics such as balance or memory and was closer to the animal head impact location, it was hypothesized that a larger deficit in the corresponding concussion metric would be observed. Although this still may be true, the results of the study can be attributed to several different reasons.

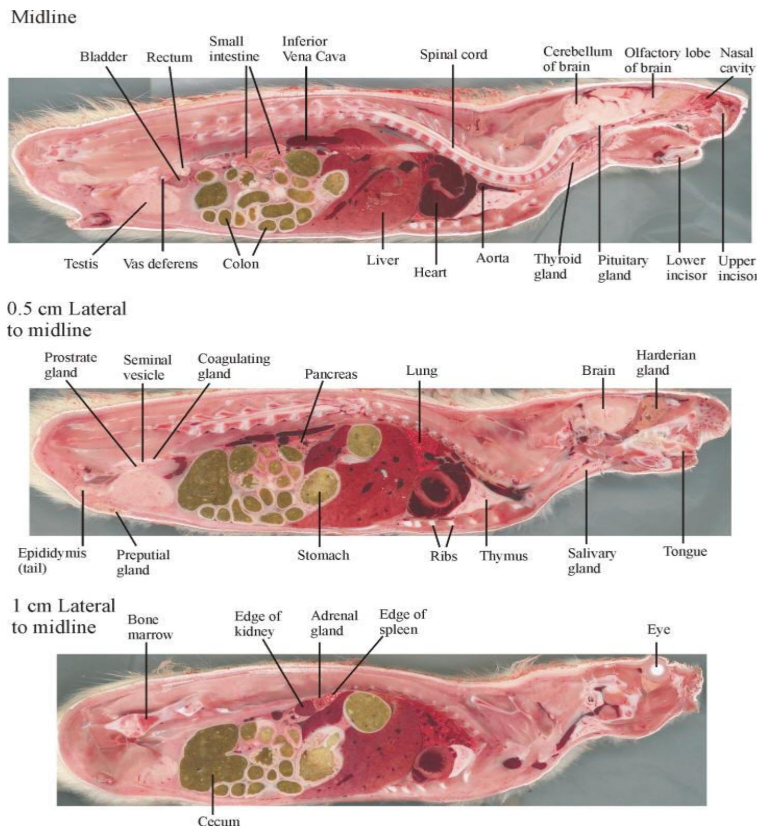

Figure 6: microPET of rat cross section that displays major anatomical features.

Figure 6 reveals the major anatomical structures of a rat using microPET and audioradiography. As noted in the first scan, the amount of space between the exterior of the head and the brain increases as you move from the top of the head towards the rear. This space contains tissue that can potentially act to dissipate impact forces to the head. The back impacts may have been located far back enough along the head of the rat to encounter significant amounts of tissue that reduced and dissipated the impact force. This dissipation could have also reduced jarring motion of the brain, which is a significant cause of brain damage during mild brain injuries (15). Force applied by the CCI was reduced from the parameters in the paper in order to prevent skull fracture. Though the impact may have produced a deficit in the top of the head, the increased distance between the skull and the brain (which is covered by soft tissue) at the back may have dissipated some of the force and reduced the jarring impact on the brain.

$$\varepsilon = \frac{l_0 - l_i}{l_0}$$

ε = strain (deformation of skull)
l_0 = initial length before impact
l_i = final length after impact

$$\varepsilon = \frac{0.0015\text{m}}{l_0}$$

$$\varepsilon = \frac{0.001\text{m}}{l_0}$$

Strain is smaller with reduced impact depth. The reduced deformation helps prevent fracture of the skull. Since stress is directly proportional to strain, the reduced strain results in reduced stress on the rat head. This helps prevent skull fracture. The extra cushioning between the brain and the back of the head may have reduced the stress/strain that the brain experiences within the skull, which would reduce the magnitude of injury and symptoms.

When applying the impacts with the CCI device, the force of the impact was held constant at both locations. Although the impact force may have been the same, the resultant accelerations were different due to differences in rotational acceleration, which is dependent on the location and direction of the applied force (31). Given the different impact locations on the rat head, the acceleration produced at the back of the head may have been lower than at the top, resulting in reduced damage and a lack of significant behavioral deficits at this location.

It is possible that the tests employed in the study were not sensitive enough to detect any behavioral changes that may have been present. In addition to memory, structures in the back of the brain are linked to vision. The impacts to the head were intended to cause concussion symptoms such as disorientation, loss of balance, and loss of memory as seen in football players who sustain substantial hits to the head, but is possible that the post-injury tests were not sensitive enough to pick up any changes pertaining to these symptoms. It is possible that the impacts primarily caused other types of concussion related deficits such as weakening of vision that can be more accurately screened for by using alternate post-injury examinations such as the visual cliff and elevated plus maze, the Morris water maze, Lashley jump stand, the Eight-arm radial arm maze, and the Shuttle box. Future studies may employ the use of these alternate post-injury tests.

Concussions are not solely characterized by changes in observable behavior. These behavioral changes are attributed to traumatic axonal injuries (TAI) (28). It is possible that these notable microstructural differences in the brain exist following injury that can only be measured through brain imaging techniques. Future studies may employ the use of these techniques that can reveal microscopic changes in the brain. Although traditional scanning techniques such as CT and MRI scans cannot reveal such injuries, Diffusion Tensor Imaging (DTI) has been used to detect brain abnormalities in mTBI (28). DTI measures microscopic changes in white matter tracts of the brain by measuring the restriction of water diffusion. It functions on the premise that microstructural changes (changes in fibers and various macromolecules), as a result of injury, will create obstacles to water movement. It is sensitive enough to inspect specific tracts of the brain such as the corpus callosum to determine the degree of damage due to mTBI.

There is also the minute possibility that injuries sustained to the top of the head may be more detrimental to post-injury brain function than impacts to the back of the head when it comes to balance. Although the study was intended to produce measurable concussion symptoms for both locations for comparison, it is possible that injuries to the back of the head may require a higher amount of force to result in a concussion. Of course, this cannot be statistically proven unless both injuries are measurable.

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